AR201-13465B

BOILING POINT

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 96.51%

Test Method: OECD Method 103, EEC Method A2

GLP: Yes

Year Performed: 2001

Laboratory: Huntingdon Life Sciences

Results: 266.5 °C – 322 °C

Data Quality: 1, Reliable without restriction

References: Chlorendic Anhydride. Boiling Point. Huntingdon Life Sciences. 2001.

PARTITION COEFFICIENT

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 96.51%

Test Method: OECD Method 107, EEC Method A8

GLP: Yes

Year Performed: 2001

Laboratory: Huntingdon Life Sciences

Remarks: Chlorendic anhydride hydrolyses on contact with water to chlorendic acid making it impossible to perform the study on the anhydride. The test was therefore performed using chlorendic acid to obtain an environmentally relevant result.

Results: Chlorendic acid Log P = 1.39 at 20 °C

Data Quality: 1, Reliable without restrictions

References: Chlorendic Anhydride. Partition Coefficient. Huntingdon Life Sciences. 2001.

WATER SOLUBILITY

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 96.51%

Test Method: OECD Method 105, EEC Method A6

GLP: Yes

Year Performed:	2001
Laboratory:	Huntingdon Life Sciences
Remarks:	It was found in early development that chlorendic anhydride hydrolyses to the acid form on contact with water and is therefore not possible to determine its water solubility. It was decided that the solubility with respect to the acid would be determined and therefore calibrated against the acid.
Results:	The analytical data yielded a range of water solubility of 0.454 to 0.508 g/l and a pH range of 3.12 to 3.25. The overall mean water solubility is 0.49932 g/l with a standard deviation of 0.037496 g/l. The test solutions were also analyzed against chlorendic anhydride standards to check for the presence of the unhydrolyzed test substance. No peaks were observed in the test solutions at the retention time of chlorendic anhydride. The limit of detection for chlorendic anhydride is 2.5×10^{-3} g/l and hence the solubility of chlorendic anhydride in its unhydrolyzed form is less than this value.
Conclusion:	Chlorendic acid 0.499 g/l at 20°C Chlorendic anhydride in its unhydrolyzed form is $< 2.5 \times 10^{-3}$ g/l
Data Quality:	1, Reliable without restrictions
References:	Chlorendic Anhydride. Water Solubility. Huntingdon Life Sciences. 2001.

ENVIRONMENTAL FATE

STABILITY IN WATER (HYDROLYSIS)

Anhydrides are a well-known class of compound, formed by the removal of one molecule of water from two molecules of carboxylic acids. If the carboxylic acid groups are present in the same molecule, a cyclic anhydride, such as chlorendic is produced.

Anhydrides are well known to react with water and revert to the dicarboxylic acid. This means that anhydrides do not usually exist in water but react with it and dissolve as the parent acid. Work on the experimental determination of the water solubility of chlorendic anhydride has confirmed that it too dissolves only as the acid with no anhydride being found in the aqueous solution. It can be concluded that, on dissolving in water, chlorendic anhydride undergoes immediate hydrolysis to the corresponding di-acid. Therefore, conducting the hydrolysis test is not necessary.

TRANSPORT (FUGACITY)

The fate and behavior of chlorendic anhydride and its hydrolysis product, chlorendic acid, in a model environment consisting of four main compartments, air, water, soil and sediment, have been evaluated using the Mackay Level III Fugacity Model, version 2.20.

Inputs to the Model:

Chlorendic Anhydride

Physical Chemistry Properties

Chemical Type	1	A chemical that partitions into all media
Molecular mass	370.83	Molecular formula $C_9H_2Cl_6O_3$
Data Temperature	25°C	
Log K_{ow}	4.37	Estimation
Water Solubility (g/m^3)	0.0982	Estimation
Vapor Pressure (Pa)	9.47×10^{-3}	Estimation
Melting Point	233°C	Not known

Half-Lives

Half-life in Air	23.521 hours
Half-life in Water	2.78×10^{-3} hours
Half-life in Soil	2.78×10^{-3} hours
Half-life in Bulk Sediment	2.78×10^{-3} hours
Half-life in Suspended Sediment	2.78×10^{-3} hours
Half-life in Fish	2.78×10^{-3} hours
Half-life in Aerosol	2.78×10^{-3} hours

Dimensions and Other Properties

The parameters that define the model environment are:
Volume of each environmental compartment (m^3)
Density of each environmental compartment (kg/m^3)
Organic carbon content of soil and sediments (g/g)
Lipid content (kg/m^3)
Transport velocities between compartments (m/h)

Emissions to the Model Environment

Chlorendic anhydride emitted to either the water or soil compartments will hydrolyze rapidly to the acid resulting in extremely low environmental concentrations of the parent substance. Therefore, the model has been run assuming 100% emission to air.

Chlorendic Acid

Physical Chemistry Properties

Chemical Type	1	A chemical that partitions into all media
Molecular mass	388.85	Molecular formula $C_9H_4Cl_6O_4$
Data Temperature	25°C	
Log K_{ow}	1.32	OECD 107
Water Solubility (g/m^3)	499	OECD 105
Vapor Pressure (Pa)	4.053×10^{-6}	Estimation
Melting Point	181.33°C	Estimation

Half-Lives

Half-life in Air	15.688 hours
Half-life in Water	4320 hours
Half-life in Soil	4320 hours
Half-life in Bulk Sediment	4320 hours
Half-life in Suspended Sediment	4320 hours

Half-life in Fish 4320 hours
Half-life in Aerosol 15.688 hours

Dimensions and Other Properties
The parameters that define the model environment are:
Volume of each environmental compartment (m^3)
Density of each environmental compartment (kg/m^3)
Organic carbon content of soil and sediments (g/g)
Lipid content (kg/m^3)
Transport velocities between compartments (m/h)

Emissions to the Model Environment
Because no information was available on probable real-life emissions of chlorendic anhydride (and hence the acid) into the environment the recommendation of Mackay et al (1996) was followed: that is the model was run for 1000 kg/h emissions to each of the air, water and soil compartments individually and then in total. This standardized approach allows comparison with other compounds and provides information on the main source of the chemical in each compartment.

Results:

Chlorendic Anhydride
Using the Level III program and with emissions of 1000 kg/h to the air compartments, the model estimated the following distribution:

Compartment	Amount	Concentration	Loss through advection	Loss through reaction
Air	100.0%	148 ng/m^3	14.8%	84.8%
Soil	<0.1%	6.12×10^{-5} ng/l	<0.01%	0.305%
Water	<0.1%	6.13×10^{-8} ng/g	-	0.0412%
Sediment	<0.1%	5.83×10^{-12} ng/g	<0.01%	<0.01%

The predominant routes of loss were by reaction in, and advection from, the air compartment. The estimated mean residence time for chlorendic anhydride in the model environment (persistence) was 14.8 hours.

Chlorendic Acid
Using the Level III program and with emissions of 1000 kg/h to each of the air, water and soil compartments, the model estimated the following distribution:

Compartment	Amount	Concentration	Loss through advection	Loss through reaction
Air	<0.1%	0.234 ng/m^3	<0.01%	0.0345%
Soil	42.9%	10913 ng/l	72.8%	11.7%
Water	57.0%	107 ng/g	-	15.5%
Sediment	0.096%	7.65 ng/g	<0.01%	0.0262%

The predominant routes of loss were by reaction in the soil, and water compartments and advection from the water compartment. The estimated mean residence time for chlorendic acid in the model environment (persistence) was 1696 hours.

Conclusion: Chlorendic anhydride: In aqueous solution chlorendic anhydride rapidly hydrolyses to the acid. Simulations in which the anhydride was emitted to the water or soil compartments would merely show rapid disappearance due to hydrolysis. Therefore the only simulation run was for emission to air. Under these conditions nearly all (> 99.9 %) of the anhydride in the system was in the air compartment. Losses from the system were high and the mean residence time was only 14.8 hours. The simulation results probably overestimate

concentrations in air because no account was taken of reaction with atmospheric water.

Chlorendic acid: As expected the simulation results showed that the acid was much more persistent in the environment than the anhydride. They also showed deposition from air onto soil and water, transport from soil to water but little deposition from water into the sediment compartment. The primary route of loss from the system was advection from the water compartment but degradation in the soil and water compartments was also significant. There were a number of uncertainties regarding the values used for model inputs so the results should be treated with appropriate caution.

Reliability: Estimated values based on accepted model.

References: Chlorendic Anhydride. Estimation of Environmental Fate Using the Mackay Level III Fugacity Model. Huntingdon Life Sciences. 2001.

BIODEGRADATION

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 95-100%

Test Method: OECD Procedure 302C and OECD Procedure 301F

GLP: Yes

Test Type: Inherent biodegradability, manometric respirometry

Year Performed: 2001

Laboratory: Huntingdon Life Sciences

Inoculum: Activated sludge from sewage treatment works (100 mg solids/liter)

Control and blank: Inoculated mineral salts medium

Positive Control: Inoculated mineral salts medium plus sodium benzoate

Temperature and pH: The temperature of the water bath ranged from 20.7 to 24.8°C. The pH of the biotic test mixtures ranged between 7.0 and 7.2 at the start of the test and 6.0 and 7.9 at the end of the test.

Duration: 32 Days

Study Design: Test and control mixtures were prepared according to the following schedule: In each case, the final volume was 550 ml.

Flask	Contents
1 and 2	Control – Inoculated mineral salts medium ("MSM")
3	Reference – Inoculated MSM + sodium benzoate (100 mg/l)
4 and 5	Test – Inoculated MSM + test substance (162.3 mg/l)
6	Inhibition assay – Inoculated MSM + test substance (162.3 mg/l)
7	Abiotic control – Ultrapure water + test substance (162.3 mg/l)
8	Ultrapure water alone

The potential inhibitory effect of chlorendic anhydride on the degradative activity of the microbial inoculum was assessed by examining the rate and extent of degradation of sodium benzoate (100 mg/l) in the presence of chlorendic anhydride (162.3 mg/l). Degradation of the test or reference substance was expressed as the cumulative amount of oxygen consumed as a percentage of the respective Theoretical Oxygen Demand (ThOD).

The data recording system was programmed to record the amount of oxygen generated in units of 0.4 mgO_2.

Results: The ThOD values of the mixtures containing the test substance alone and with sodium benzoate were calculated to be 50.0 mgO_2 and 141.9 mgO_2 respectively; the ThOD of the mixture containing sodium benzoate alone was 91.9 mgO_2.

Sodium benzoate was degraded by 64.6% after 3 days and 87.7% after 28 days of incubation; no further biodegradation had taken place by Day 31. In the presence of chlorendic anhydride, degradation of sodium benzoate had achieved 61.9% on Day 2 and 83.8% at the end of Day 31. Cumulative levels of oxygen consumption by the biotic control mixtures after 31 days were 30.0 and 33.6 mgO_2. These results confirm that chlorendic anhydride was not inhibitory to the activity of the microbial inoculum and that the test was valid.

The level of oxygen consumption by the biotic mixtures containing chlorendic anhydride was negligible by Day 31. Oxygen consumption by the abiotic mixture containing chlorendic anhydride was also negligible (2.4% of the ThOD).

Conclusions: There was no evidence of biodegradation of chlorendic anhydride by the end of the test on Day 31. The oxygen consumption by the abiotic mixture of chlorendic anhydride was also negligible (equivalent to approximately 2.4% of the ThOD). Therefore, chlorendic anhydride can not be considered to be inherently biodegradable. The results obtained for the degradation of sodium benzoate in the presence of chlorendic anhydride (61.9% after 2 days) confirmed that the inoculum was viable and that its activity was not affected by the presence of the test substance.

Data Quality: 1, Reliable without restrictions

References: Chlorendic Anhydride. Assessment of Inherent Biodegradability By Manometric Respirometry. Huntingdon Life Sciences. 2001.

ACUTE TOXICTY TO FISH

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 93.81%

Test Method: Test practices followed those recommended by the United States Environmental Protection Agency's Committee on Methods for Toxicity Test with Aquatic Organisms with the exception that replicate concentrations were not used.

Committee on Methods for Toxicity Test with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macro-invertebrates and Amphibians. EPA-660 / 3-75-009. 1975.

Test Type: Acute toxicity to fish

GLP: No

Laboratory: Union Carbide Environmental Services

Year Performed: 1977

Species/Strain: *Salmo gairdneri* Richardson

Analytical Monitoring: Dissolved oxygen and pH were determined initially and every 48 hours thereafter for the control, high, medium and low toxicant concentrations, while temperature was determined initially and at the termination of the test for above. In addition to obtaining the above chemical and physical parameters, abnormal behavioral responses of the test fish were noted and recorded at 24-hour intervals. Also, determination of test material concentration was taken at 24, 48 and 96 hours.

Exposure Period: 96 Hours

Test Details: Static

Statistical Methods: LC_{50} values and 95% confidence limits were determined at the 24, 48 and 96 hour exposure periods by the Spearman-Karber Estimator

Finney, D.J., Statistical Method in Biological Assay. 2nd Edition. 1971.

Test Condition Remarks:

Fish Size and Age: Mean length 44 mm; Mean weight 0.61 grams; Age 3 months old

Test Conditions:

pH: 7.54	Total Alkalinity: 28 mg/l as $CaCO_3$
Total Hardness: 42 mg/l as $CaCO_3$	Specific Conductivity: 145 μmhos/cm

Diluent Water Source and Chemistry: Dilution water was obtained from a well and treated with a Continental Reverse Osmosis Water System and deionized. After treatment, the water is reconstituted to the desired pH and hardness according to the procedures of Marking and Dawson.

Marking, L.L. and V.K. Dawson. Toxicity of Quinaldine Sulfate to Fish. Invest. Fish Control No. 48. U.S. Fish and Wildlife Service. 1973.

Stock and Test Solutions: The chemical was weighed and added directly to 15 liters of soft water in the test vessels. The test was started by introducing the toxicant into test vessels containing dilution water, thoroughly mixing, and then introducing the fish. A slight precipitate was noted for all concentrations despite vigorous mixing.

Vessels: 5 gallon, chemically clean, glass jars containing 15 liters of water

Fish per Vessel: 10

Dose Selection: 0, 100, 180, 320, 560, and 1000 mg/l

Temperature range: 10.0°C +/- 1°C

pH range: 7.5 - 9.3

Analytical Results:

Percent Mortality

Chlorendic Anhydride Nominal Concentration (mg/l)	Control	100.0	180.0	320.0	560.0	1000.0
24 Hour	0%	0%	0%	0%	0%	10%
48 Hour	0%	0%	0%	0%	0%	60%
96 Hour	0%	0%	0%	0%	100%	100%

LC_{50} Values

		24 Hour	48 Hour	96 Hour
LC_{50} (mg/l)		>1000.0	947.6	422.7
95%Confidence Interval	**Low**	NA	785.0	NA
	High	NA	1144.0	NA

NA = Not available due to a lack of partial kills

Results: The 96-hour LC_{50} for chlorendic anhydride to rainbow trout is 422.7 mg/l. Behavioral observations made during the test indicated that rainbow trout exposed to concentrations of 100.0 mg/l and higher became excitable with sounding and dark discoloration observable. In addition, some animals were seen to be covered with the material. Consequently, the no-observable-effect level was <100 ppm.

Conclusions: The 96-hour LC_{50} of chlorendic anhydride to rainbow trout is 422.7 mg/l. This value is based upon nominal concentrations of the compound in soft reconstitued water.

Data Quality: 2, Valid with restriction

References: The Acute Toxicity of Chlorendic Anhydride to the Rainbow Trout, *Salmo gairdneri* Richardson. Union Carbide Environmental Services. 1977.

ACUTE TOXICTY TO FISH

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 93.81%

Test Method: Test practices followed those recommended by the United States Environmental Protection Agency's Committee on Methods for Toxicity Test with Aquatic Organisms with the exception that replicate concentrations were not used.

Committee on Methods for Toxicity Test with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macro-invertebrates and Amphibians. EPA-660 / 3-75-009. 1975.

Test Type: Acute toxicity to fish

GLP: No

Laboratory: Union Carbide Environmental Services

Year Performed: 1977

Species/Strain: *Lepomis macrochirus* Rafinesque

Analytical Monitoring: Dissolved oxygen and pH were determined initially and every 48 hours thereafter for the control, high, medium and low toxicant concentrations, while temperature was determined initially and at the termination of the test for above. In addition to obtaining the above chemical and physical parameters, abnormal behavioral responses of the test fish were noted and recorded at 24-hour intervals. Also, determination of test material concentration was taken at 24, 48 and 96 hours.

Exposure Period: 96 Hours

Test Details: Static

Statistical Methods: LC_{50} values and 95% confidence limits were determined at the 24, 48 and 96 hour exposure periods by the Spearman-Karber Estimator

Finney, D.J., Statistical Method in Biological Assay. 2nd Edition. 1971.

Test Condition Remarks:

Fish Size and Age: Mean length 27 mm; Mean weight 0.19 grams; Age 3 months old

Test Conditions:

pH: 7.40	Total Alkalinity: 28 mg/l as $CaCO_3$
Total Hardness: 42 mg/l as $CaCO_3$	Specific Conductivity: 145 μmhos/cm

Diluent Water Source and Chemistry: Dilution water was obtained from a well and treated with a Continental Reverse Osmosis Water System and deionized. After treatment, the water is reconstituted to the desired pH and hardness according to the procedures of Marking and Dawson.

Marking, L.L. and V.K. Dawson. Toxicity of Quinaldine Sulfate to Fish. Invest. Fish Control No. 48. U.S. Fish and Wildlife Service. 1973.

Stock and Test Solutions: Individual amounts of the toxicant were weighed to a precision of 0.1 mg and added directly to the test vessels. The test was started by introducing the toxicant into test vessels containing dilution water, thoroughly mixing, and then introducing the fish. A slight precipitate was noted for all concentrations despite vigorous mixing.

Vessels: 5 gallon, chemically clean, glass jars containing 15 liters of water

Fish per Vessel: 10

Dose Selection: 0, 100, 180, 320, 560, and 1000 mg/l

Temperature range: 22.0°C +/- 1°C

pH range: 7.40 - 9.2

Analytical Results:

Percent Mortality

Chlorendic Anhydride Nominal Concentration (mg/l)	Control	100.0	180.0	320.0	560.0	1000.0
24 Hour	0%	0%	0%	0%	0%	60%
48 Hour	0%	0%	0%	0%	10%	100%
96 Hour	0%	0%	0%	0%	100%	100%

LC_{50} Values

		24 Hour	48 Hour	96 Hour
LC_{50} (mg/l)		>560.0	710.3	422.7
95%Confidence Interval	**Low**	NA	632.9	NA
	High	NA	797.1	NA

NA = Not available due to a lack of partial kills

Results: The 96-hour LC_{50} for chlorendic anhydride to bluegill sunfish is 422.7 mg/l. Behavioral observations made during the test indicated that bluegill sunfish exposed to concentrations of 560.0 mg/l and higher died while 320.0 mg/l and lower no abnormal behavior was observed. Mortalities at the higher concentrations may be due to

suffocation (some animals were observed to be covered by the material). Consequently, the no-observable-effect level was 320 ppm.

Conclusions: The 96-hour LC_{50} of chlorendic anhydride to bluegill sunfish is 422.7 mg/l. This value is based upon nominal concentrations of the compound in soft reconstitued water.

Data Quality: 2, Valid with restriction

References: The Acute Toxicity of Chlorendic Anhydride to the Bluegill Sunfish, *Lepomis macrochirus* Rafinesque. Union Carbide Environmental Services. 1977.

ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 93.81%

Test Method: Test practices followed those recommended by the United States Environmental Protection Agency's Committee on Methods for Toxicity Test with Aquatic Organisms.

Committee on Methods for Toxicity Test with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macro-invertebrates and Amphibians. EPA-660 / 3-75-009. 1975.

Test Type: Acute *Daphnia* immobilization test

GLP: No

Year Performed: 1977

Laboratory: Union Carbide Environmental Services

Species/Strain: *Daphnia magna*

Analytical Monitoring: Determination of test material concentration at 24 and 48 hours

Exposure Period: 48 hours

Test Details: Static without renewal

Statistical Methods: LC_{50} values and 95% confidence limits were determined by the Spearman-Karber Estimator.

Finney, D.J., Statistical Method in Biological Assay. 2nd Edition. 1971.

Test Condition Remarks:

Age at Initiation:	Less than 20 hours	
Test Conditions:	Water quality:	Soft
	Hardness:	52 mg/l as $CaCO_3$
	Alkalinity:	29 mg/l $CaCO_3$
	Specific Conductivity:	130 µmhos/cm

Solvent:	Acetone
Vessel:	250 ml glass beakers
Daphnids per Vessel:	5 organisms per test beaker
Dose Selection:	Control, Solvent control, 18, 32, 56, 100, 180 mg/l
Temperature range:	17 +/- 2°C
Solution pH range:	4.50 - 7.43
Dissolved Oxygen:	6.2 – 9.1

Analytical Results:

Percent Mortality

Chlorendic Anhydride Nominal Concentration (mg/l)	Control	Solvent Control	18.0	32.0	56.0	100.0	180.0
24 Hour	0%	0%	0%	0%	0%	10%	100%
48 Hour	0%	0%	0%	0%	0%	35%	100%

LC_{50} Values

		24 Hour	48 Hour
LC_{50} (mg/l)		127.9	110.7
95%Confidence Interval	**Low**	118.1	97.6
	High	138.4	125.6

Results: The 48-hour LC_{50} for chlorendic anhydride to *Daphnia magna* is 110.7 with 95% confidence limits of 97.6-125.6 mg/l.

Conclusions: The 48 hour LC_{50} is 110.7 mg/l and the No Observed Effect Level is 56.0 mg/l.

Data Quality: 2, Valid with restriction

References: Acute Toxicity of Chlorendic Anhydride to the Water Flea *Daphnia Magna* Straus. Union Carbide Environmental Services. 1977.

ACUTE TOXICITY TO AQUATIC PLANTS

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 95-100%

Test Method: OECD Guideline 201

Test Type: Algal Inhibition Test

GLP: Yes

Date Performed: 2001

Laboratory: Huntingdon Life Sciences

Species: *Selenastrum capricornutum,* Strain number CCAP 278/4

Element basis: Area under the curve (72 hours), growth rate (0-72 hours)

Exposure period: 72 hours

Test substance: An aqueous stock dilution of the test substance was prepared at a nominal concentration of 100 mg/l by adding chlorendic anhydride (100 mg) to nutrient medium (1 l). To aid dissolution, it was treated with ultrasound for twenty minutes and stirred vigorously in darkness overnight in the test area. This dilution was either used directly at the highest test concentration or serially diluted to provide the test media at the lower concentrations. An aliquot (2.8 ml) of algal inoculum was then added to the test medium (400 ml) at each concentration to give an initial cell density of $1x10^4$ cell/ml. Approximately 100 ml of the appropriate inoculated test medium was added to each flask.

Control Cultures: The nutrient medium control cultures were prepared by inoculating culture medium (800 ml) with algal inoculum (5.6 ml); approximately 100 ml were placed into each control vessel.

Test Conditions:

Test Temperature Range: 23.6 to 24.8°C

Exposure Vessel Type: 250 ml conical flask each containing 100 ml of test or control culture were placed in an illuminated orbital incubator according to a random number sequence.

Light levels and quality during exposure: The cultures were incubated, without medium renewal for 72 hours under continuous illumination of approximately 7300 lux provided by 6X30 W "cool white" 1 meter fluorescent tubes with an intensity setting of 100%.

Test Design:

Number of replicates: Five exposure levels were prepared plus one untreated control, each in triplicate

Nominal Initial Loading Rates: 6.25, 12.5, 25, 50, and 100 mg chlorendic anhydride /l

Equivalent to: 6.56, 13.1, 26.3, 52.5 and 105 mg chlorendic acid /l

Analysis: The exposure levels were monitored by measuring the concentrations of chlorendic acid using a HPLC method of analysis.

Analytical Results:

Nominal Concentration (mg/L)		Measured Chlorendic Acid Concentrations (mg/L)					
Chlorendic Anhydride	Chlorendic Acid	0 Hours	% N	72 Hours	% N	% ti	Overall mean
0	0	ND	-	ND	-	-	-
6.25	6.56	6.44	98	6.69	102	104	6.57
6.25 A	6.56 A	-	-	6.54	100	102	-
12.5	13.1	13.4	102	12.9	98	96	13.2
25	26.3	26.1	99	24.5	93	94	25.3
50	52.5	47.9	91	48.9	93	102	48.4
100	105	98.9	94	95.5	91	97	97.2
100 A	105 A	-	-	101	96	102	-

ND None detected (Limit Of Detection: 0.01 mg chlorendic acid / L
% N Measured concentration expressed as a percentage of the nominal concentration
% ti Measured concentration after 72 hours expressed as a percentage of the starting concentration
A Culture medium incubated under test conditions without algal cells

Results:

Chemical analysis

The measured levels of chlorendic acid in samples of the test cultures taken at the start and end of the test ranged between 91 and 102% of their nominal values, giving overall mean measured levels of 6.57, 13.2, 25.3, 48.4 and 97.2 mg/l.

After 72 hours, analysis of samples of media containing the test substance at the lowest and highest exposure levels, which had been incubated without algal cells, gave similar results to test media incubated in the presence of algal cells, suggesting that the presence of algae had not affected the stability of the hydrolysis product chlorendic acid under the conditions of the test.

Algal growth

Algal growth test results have been expressed in terms of mean measured concentrations of chlorendic acid. The following values were derived from the data:

Area under the growth curve:
E_bC_{50} (72 h) >97.2 mg/l (46% inhibition)

Average specific growth rate
E_rC_{50} (0-72 h) >97.2 mg/l (13% inhibition)

"NOEC" 48.4 mg/l

Conclusions: After 72 hours of exposure to the test substance (added as chlorendic anhydride), neither the E_bC_{50} nor E_rC_{50} could be identified because algal growth had not been inhibited but both were considered to be greater than 97.2 mg chlorendic acid / l (measured).

The "No Observed Effect Concentration" (NOEC) for both area under the growth curve and growth rate was 48.4 mg chlorendic acid / l (measured).

Data Quality: 1, Reliable without restrictions

References: Chlorendic Anhydride. Algal Growth Inhibition Assay. Huntingdon Life Sciences. 2001.

ACUTE TOXICTY

ORAL

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 93.81%

Study Type: Acute oral toxicity

Test Method: The rats were housed by sex, in groups of 5 rats per cage, in hanging wire-mesh cages in temperature and humidity controlled quarters. They were maintained in accordance with the recommendations contained in the H.E.W. Publication No. 74-23 (N.I.H.) entitled "Guide for the Care and Use of Laboratory Animals". Water and Purina Laboratory Chow were available except for an overnight period immediately preceding oral administration during which food, but not water, was withheld. The test material was suspended in corn oil and administered orally by gavage at the following dosage levels to male and female rats: 807.1, 1281, 2034, 3229, 5126 mg/kg. Five rats of each sex were used at each dosage level. Volumes of 10 ml/kg of body weight were administered at all dosage levels. All rats were observed for mortality and pharmacotoxic signs during the first four hours after dosing, at 24 hours and daily thereafter for a total of 14 days. Body weights were recorded immediately prior to dosing (control weight) and at 7 and 14 days. All rats that died in the study were subjected to gross necropsy examination.

GLP: No

Year Performed: 1978

Laboratory: International Research and Development Corporation

Species/Strain: Rat, Charles River CD

Sex: Male and Female

Results: Acute oral LD_{50} and 95% Confidence Limits
Male rats: 2562 (2218-2961) mg/kg
Female rats: 2130 (1698-2671) mg/kg
Combined male and female rats: 2336 (2065-2643) mg/kg

Number of Deaths at Each Dose Level:

2034 mg/kg	2 Female
3229 mg/kg	5 Male, 5 Female
5126 mg/kg	5 Male, 5 Female

Time of Death of Each Animal:

Day 1	
5126 mg/kg	3 male, 4 female
3229 mg/kg	1 male
Day 2	
5126 mg/kg	2 male, 1 female
3229 mg/kg	2 male, 5 female
2034 mg/kg	1 female
Day 3	
3229 mg/kg	2 male
Day 5	
2034 mg/kg	1 female

Description of Clinical Effects:

At 807.1 mg/kg, 2 of 5 males and 1 of 5 females displayed diarrhea up to 4 hours after dosing.

At 1281 mg/kg, 4 of 5 males displayed diarrhea 2.5 hours after dosing and 3 of 5 males displayed diarrhea up to 4 hours after dosing. All 5 rats were normal, in this respect, during days 1-14.

At 1281 mg/kg, 3 of 5 female rats showed diarrhea 1 hour after dosing and 2 of 5 up to 4 hours after dosing. All 5 rats were normal, in this respect, during days 1-14.

At 2034 mg/kg, diarrhea was seen in 1 of 5 male rats up to 4 hours after dosing. Similarly in females, 1 of 5 rats displayed diarrhea up to 4 hours after dosing. Additionally, the following results were seen in female rats at this dose level: Day 2, one rat died; Day 4, one rat was displayed hypoactivity and one displayed ataxia; Day 5, one rat died; and on days 6-11, one rat displayed alopecia.

At 3229 mg/kg, in males, after one hour diarrhea was seen in 3 of 5 rats and 3 of 5 rats showed hypoactivity. After 4 hours, 5 of 5 rats showed diarrhea and hypoactivity. On day 1, 1 animal showed a urine stained abdomen and one animal died. On day 2, 2 rats died and on day 3, the remaining two rats died. For females at this dose level, after 1 hour, diarrhea was seen in 2 of 5 rats and hypoactivity in 3 of 5 rats. After 4 hours, all five animals displayed diarrhea and hypoactivity. On day 1 2 of 5 rats displayed a urine stained abdomen. On day 2, all five animals died.

At 5126 mg/kg, in males 4 hours after dosing, 1 of 5 rats displayed diarrhea and 2 of 5 showed hypoactivity. On day 1, 2 of 5 showed hypoactivity, 2 of 4 showed decreased limb tone, 1 of 5 displayed prostration and 3 of 5 died. On day 2, the remaining 2 rats died. For females at this dosage level, after four hours, 1 of 5 rats displayed diarrhea and 2 of 5 showed hypoactivity. On day 1, one rat displayed hypoactivity, one displayed decreased limb tone, one showed tremors and 4 of the 5 rats died. On day 2, the final rat died.

Necropsy findings: The following effects were seen by a number of rats exhibiting signs / a number of rats necropsied at specified dose levels:

Yellow stained urogenital region: 1/2 females at 2034 mg/kg and 2/4 females at 3229 mg/kg

Congested lungs: 2/2 females at 2034 mg/kg, 3/5 males at 3229 mg/kg, 3/4 females at 3229 mg/kg

Stomach, fluid filled: 1/2 females at 2034 mg/kg, 1/5 males at 5126 mg/kg and 5/5 females at 5126 mg/kg

Stomach, distension: 3/5 males at 3229 mg/kg, 2/4 females at 3229 mg/kg, 5/5 males at 5126 mg/kg and 5/5 females at 5126 mg/kg

Stomach, glandular mucosa, leathery in texture: 2/4 females at 3229 mg/kg, 2/5 males at 5126 mg/kg and 3/5 females at 5126 mg/kg

Stomach, glandular mucosa, focal hemorrhage: 1/5 males at 5126 mg/kg

Stomach, mucosa, hyperemia: 2/2 females at 2034 mg/kg, 1/5 males at 3229 mg/kg, 2/4 females at 3229 mg/kg and 2/5 males at 5126 mg/kg

Stomach, glandular mucosa, erosion: 1/5 males at 3229 mg/kg, 1/5 males at 5126 mg/kg and 1/5 females at 5126 mg/kg

Red moist fluid around nose: 1/2 females at 2034 mg/kg, 1/4 females at 3229 mg/kg

Thymus, congestion and focal hemorrhage: 1/2 females at 2034 mg/kg, 2/5 males at 3229 mg/kg, 3/4 females at 3229 mg/kg and 2/5 males at 5126 mg/kg

No gross lesions: 1/5 males at 3229 mg/kg

Data Quality: 2, Valid with restriction

References: Chlorendic Anhydride. Acute Toxicity Studies in Rabbits and Rats. International Research and Development Corporation. 1978.

DERMAL

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 93.81%

Study Type: Acute dermal toxicity

Test Method: Rabbits were divided into two groups containing 2 male and 2 female rabbits each. The hair was removed from the back of each rabbit (20-30% of the body surface) with an electric clipper. The skin of one male and one female in each group was abraded with a scalpel blade. The abrasions penetrated the stratum corneum, but were not sufficiently deep to cause bleeding. The test material was applied once only to the backs of the rabbits at the following dosage levels: 10000 and 20000 mg/kg. The area of application was wrapped with gauze bandaging and occluded with Saran Wrap. Twenty-four hours later the bandages were removed and the backs were washed with tepid tap water. They were observed at 24 hours and daily thereafter for a total of 14 days for mortality, pharmacotoxic signs and dermal irritation. Body weights were measured

immediately preceding dosing and at 7 and 14 days. All rabbits that died in the study were subjected to gross necropsy examination.

GLP: No

Laboratory: International Research and Development Corporation

Year Performed: 1978

Species/Strain: New Zealand white rabbits

Sex: Male and female

Results: Both male rabbits at the 20000 mg/kg dosage level died during the 14-day observation period. The minimum lethal dose by the dermal route of exposure was found to be less than 20000 mg/kg but greater than 10000 mg/kg.

Number of Deaths at Each Dose Level: 2 male rabbits dosed at 20000 mg/kg

Description of Clinical Effects: The following effects were seen at 10000 mg/kg dosing:

Day 1: Slight to marked erythema in 4 / 4 rabbits; Moderate edema in 3 / 4 rabbits; Slight atonia in 2 / 4 rabbits; Very slight coriaceousness in 2 / 4 rabbits; and subcutaneous hemorrhaging in 2 / 4 rabbits.

Day 2: Slight to marked erythema in 4 / 4 rabbits; Slight to moderate edema in 2 / 4 rabbits; Slight atonia in 2 / 4 rabbits; Very slight coriaceousness in 2 / 4 rabbits; Blanching in 1 / 4 rabbits; and subcutaneous hemorrhaging in 2 / 4 rabbits.

Day 3: Very slight to marked erythema in 4 / 4 rabbits; Slight edema in 2 / 4 rabbits; Slight atonia in 2 / 4 rabbits; Very slight coriaceousness in 2 / 4 rabbits; and blanching in 1 / 4 rabbits.

Day 4: Very slight to moderate erythema in 3 / 4 rabbits; Slight edema in 2 / 4 rabbits; Slight atonia in 2 / 4 rabbits; Very slight to slight desquamation in 2 / 4 rabbits; Slight coriaceousness in 2 / 4 rabbits; and blanching in 1 / 4 rabbits.

Days 5-6: Very slight to moderate erythema in 3 / 4 rabbits; Slight edema in 2 / 4 rabbits; Slight atonia in 2 / 4 rabbits; Slight desquamation in 3 / 4 rabbits; Slight coriaceousness in 2 / 4 rabbits; and blanching in 1 / 4 rabbits.

Days 7-10: Slight to moderate erythema in 2 / 4 rabbits; Slight edema in 1 / 4 rabbits; Slight atonia in 1 / 4 rabbits; Slight to moderate desquamation in 4 / 4 rabbits; and slight coriaceousness in 2 / 4 rabbits.

Day 11: Very slight to slight erythema in 2 / 4 rabbits; Slight atonia in 1 / 4 rabbits; Slight to moderate desquamation in 4 / 4 rabbits; and slight coriaceousness in 2 / 4 rabbits.

Days 12-13: Slight erythema in 1 / 4 rabbits; Slight atonia in 1 / 4 rabbits; and slight desquamation in 3 / 4 rabbits.

Day 14: Slight erythema in 1 / 4 rabbits; Very slight atonia in 1 / 4 rabbits; and slight desquamation in 3 / 4 rabbits.

The following effects were seen at 20000 mg/kg dosing:

Day 1: Moderate erythema in 4 / 4 rabbits; Slight to moderate edema in 4 / 4 rabbits; and slight to moderate atonia in 4 / 4 rabbits.

Day 2: Moderate erythema in 4 / 4 rabbits; Slight to moderate edema in 4 / 4 rabbits; and slight atonia in 4 / 4 rabbits.

Day 3: Slight erythema in 4 / 4 rabbits; Very slight to slight edema in 4 / 4 rabbits; Slight to moderate atonia in 4 / 4 rabbits; Very slight to slight desquamation in 4 / 4 rabbits; and very slight to slight coriaceousness in 4 / 4 rabbits.

Day 4: Slight to moderate erythema in 3 / 3 rabbits; Very slight to slight edema in 3 / 3 rabbits; Slight atonia in 3 / 3 rabbits; Very slight to slight desquamation in 3 / 3 rabbits; Very slight to slight coriaceousness in 3 / 3 rabbits; and very slight to slight fissuring in 3 / 3 rabbits.

Day 5: Slight to moderate erythema in 3 / 3 rabbits; Very slight to slight edema in 3 / 3 rabbits; Slight atonia in 3 / 3 rabbits; Very slight to slight desquamation in 3 / 3 rabbits; Very slight to slight coriaceousness in 3 / 3 rabbits; and very slight to slight fissuring in 3 / 3 rabbits.

Day 6: Slight to moderate erythema in 3 / 3 rabbits; Slight edema in 3 / 3 rabbits; Slight atonia in 3 / 3 rabbits; Very slight to slight desquamation in 3 / 3 rabbits; Very slight to slight coriaceousness in 3 / 3 rabbits; and slight fissuring in 3 / 3 rabbits.

Day 7: Slight to moderate erythema in 2 / 2 rabbits; Slight edema in 2 / 2 rabbits; Slight atonia in 2 / 2 rabbits; Very slight to slight desquamation in 2 / 2 rabbits; Slight coriaceousness in 2 / 2 rabbits; and slight fissuring in 2 / 2 rabbits.

Days 8-9: Slight erythema in 2 / 2 rabbits; Slight edema in 2 / 2 rabbits; Very slight atonia in 2 / 2 rabbits; Slight desquamation in 2 / 2 rabbits; Slight coriaceousness in 2 / 2 rabbits; and slight fissuring in 1 / 2 rabbits.

Day 10: Slight erythema in 2 / 2 rabbits; Very slight to slight edema in 2 / 2 rabbits; Very slight atonia in 2 / 2 rabbits; Slight to moderate desquamation in 2 / 2 rabbits; Very slight to slight coriaceousness in 2 / 2 rabbits; and slight fissuring in 1 / 2 rabbits.

Day 11: Very slight to slight erythema in 2 / 2 rabbits; Very slight to slight edema in 2 / 2 rabbits; Very slight to slight atonia in 2 / 2 rabbits; Slight to moderate desquamation in 2 / 2 rabbits; Very slight to slight coriaceousness in 2 / 2 rabbits; and slight fissuring in 1 / 2 rabbits.

Day 12: Very slight to slight erythema in 2 / 2 rabbits; Slight edema in 1 / 2 rabbits; Slight atonia in 1 / 2 rabbits; Slight to moderate desquamation in 2 / 2 rabbits; Slight coriaceousness in 1 / 2 rabbits; and slight fissuring in 1 / 2 rabbits.

Day 13: Very slight erythema in 2 / 2 rabbits; Slight edema in 1 / 2 rabbits; Very slight atonia in 1 / 2 rabbits; Slight to moderate desquamation in 2 / 2 rabbits; Very slight to slight coriaceousness in 2 / 2 rabbits; and slight fissuring in 1 / 2 rabbits.

Day 14: Very slight erythema in 1 / 2 rabbits; Very slight atonia in 1 / 2 rabbits; and slight desquamation in 2 / 2 rabbits.

Necropsy Findings:	Lungs, congestion	1 / 2 males
	Lungs, consolidation	1 / 2 males
	Stomach, diffuse erythema and dark red foci	1 / 2 males
	Cecum mucosa, congestion	1 / 2 males
	Kidneys, pale, mottled coloration	1 / 2 males

Data Quality: 2, Valid with restriction

References: Chlorendic Anhydride. Acute Toxicity Studies in Rabbits and Rats. International Research and Development Corporation. 1978.

REPEATED DOSE TOXICITY

Test Substance: Chlorendic anhydride is a white crystalline solid

Vehicle: Administered by dietary admixture

Study type: Subacute oral toxicity in rats

Method: Chlorendic anhydride was fed in the diet at levels of 100, 500, and 2500 ppm to three groups of rats in a 90-day subacute toxicity study; there were 15 rats/sex/group. Control rats, also 15 rats/sex/group, received basal laboratory diet only. Rats used in the study were selected from a larger group of 160 animals after observation and examination during the pretest period. Any rat with physical abnormalities or signs of disease was discarded and the remaining animals were distributed among the four groups. The diets were prepared fresh weekly. Dietary samples were collected immediately after mixing and on day 7 at week 1 and at 1, 2 and 3 months of study.

Opthalmic examinations by a veterinary opthalmologist were performed on all rats once during the pretest period and again at 3 months of study. Blood and urine samples were collected randomly from five rats/sex/group at 1, 2 and 3 months of study. Samples of liver were taken from five rats/sex/group at terminal sacrifice, frozen and stored for possible future analysis. Hematological tests included: total erythrocyte count, total and differential leukocyte counts, hemoglobin concentration and hematocrit value. Biochemical tests included: fasting blood glucose, blood urea nitrogen, serum alkaline phospatase ("SAP") activity, serum glutamic oxalacetic transaminase activity and serum glutamic pyruvic transaminase activity. Urinalyses included: description of appearance; measurement of volume, pH, and specific gravity; and qualitative tests for albumin, glucose, bilirubin and occult blood; and microscopic examination of the sediment. Animal numbers and corresponding body weights were entered onto magnetic tape which was used as the data source for the randomization procedure. If the groups were not judged to be homogeneous, new randomizations were applied until homogeneity was established.

At the termination of the 90-day period of study, all rats were sacrificed using carbon dioxide gas and necropsied. Selected organs were weighed and representative tissues were preserved in buffered 10% neutral formalin.

Twenty-six tissue types* from 10 males and 10 females from the control and 2500 ppm groups were paraffin embedded, sectioned, stained with hematoxylin and eosin and examined microscopically. In addition, frozen sections of liver and kidneys from 10 male and 10 female rats from the control and treatment groups were stained for fat with oil red 0 and examined microscopically.

* Tissues examined included:

adrenals	pituitary
aorta	prostate/uterus

	brain	sternum (bone marrow)
	eye	skin/mammary gland
	heart	spinal cord
	large intestine (colon)	stomach
	small intestine (3 levels)	testis/ovary
	kidneys (2)	thymus
	liver	thyroid
	lung and bronchi	urinary bladder
	mesenteric lymph node	nerve (sciatic)
	spleen	skeletal muscle
	pancreas	any other tissues with lesions

GLP: No

Year Performed: 1980

Laboratory: International Research and Development Corporation

Species/Strain: Rat, Charles River CD

Route of Administration: Dietary

Frequency of Treatment: Continuous

Duration of Test: 13 Weeks

Dose/Concentration Levels: 0, 100, 500, 2500 ppm

Clinical Observations Performed: The rats were observed twice daily for toxicity, moribundity and mortality. Detailed observations, individual body weights and individual food consumption were also recorded weekly. Opthalmic examinations were conducted during the pretest period and at 3 months of study. Hematological and biochemical tests and urinalyses were performed at 1, 2 and 3 months of study for five rats/sex/group.

Results:

Mortality: Three high-dose females died between the 5th and 13th week of study; no other rats died during the study. Survival at week 13 was as follows:

Treatment level (ppm)	Male Survivors	Female Survivors
0 (Control)	15/15	15/15
100	15/15	15/15
500	15/15	15/15
2500	15/15	12/15

Clinical Signs: There were no signs of overt toxicity observed for the treated rats. Some incidental signs seen in a few control and/or treated rats were malaligned upper incisors, soft stools, skin lesions, hair loss, lacrimation, corneal opacity, and redness around the eye. Eye problems, such as pale coloration of the eye, decrease in size of eyeball, dilated pupil unresponsive to light, clear or white internal eye and increased distance between pupil and cornea, occurred most frequently in rats that had blood drawn via the orbital sinus technique.

Bodyweight: The mid and high-dose males and all three groups of treated females had a decreased rate of weight gain that was compound related. The group mean body weights of the mid and high dose males and high dose females were significantly less than controls at week 13. At week 13 of the study, the group mean body weights were as follows:

Treatment Level (ppm)	Group mean body weight, g	
	Male	Female
0 Control	502	285
100	509 (+1.4)	274 (-3.9)
500	464 (-7.6)	269 (-5.6)
2500	440 (-12.4)	224 (-21.4)

Food Consumption: Mid and high-dose males and high-dose females had decreased food consumption values over the 90-day study when compared with controls; however, only the food consumption of the high-dose females was significantly less than the controls. Food consumption over the entire study was as follows:

Treatment Level	Food and Compound Consumption		Compound Consumption		
(ppm)	(g/rat/day)	Male	Female	(mg/kg/day)	Male	Female
0 (Control)		25.7	18.2		-	-
100		25.8	18.3		8	8
500		24.7	18.5		39	45
2500		24.6	16.4		202	226

Ophthalmoscopy: There were no compound-related effects in the 13-week opthalmic examinations.

Hematology: No compound-related effects were observed in the results of the hematological tests. An incidental finding was the slightly elevated number of leukocytes seen for the high-dose males at 1, 2, and 3 months of study. Other occasional statistically significant values were of no physiologic importance.

Biochemistry: Both males and females treated at 2500 ppm had consistently elevated SAP activity at 1, 2 and 3 months of study. However, the only SAP values showing statistical significance were the high-dose females at 2 and 3 months of study. In addition the mid-dose females at 2 months showed statistically significant elevated SAP values. Other statistically significant changes were noted but these values were of no physiological importance. No other compound related effects were seen in the results of the biochemical tests.

Urinalysis: No compound related effects were seen in the results of the urinalyses. An incidental finding at 3 months of study was the elevated urinary pH of two of five high-dose females tested. The few statistically significant values (specific gravity) were of no physiologic importance.

Gross pathology: The three rats from the 2500 ppm group that died during the course of the study did not show any compound-related lesions. None of the rats that were sacrificed at the termination of the study had any compound-related lesions.

Organ Weight Changes: Statistically significant ($p<0.05$) decreases in the mean absolute weight of spleens of female rats at the 2500 ppm dosage level, mean absolute weight of kidneys of male rats at the 2500 ppm dosage level, mean absolute weight of hearts of male rats at all dosage levels and the female rats at the 500 ppm dosage level, were noted. In respect to the liver, statistically significant ($p<0.01$) decreases in the mean absolute and relative weights at all dosage levels were observed. Of these variations, decreases in the mean absolute weights of hearts of male rats ($p<0.05$) and in the mean absolute and relative weights of livers ($p<0.01$) at all dosage levels, appeared to be treatment related. In the absence of any significant histomorphologic changes in these organs in the test groups, these decreases probably might be due to the overall reduction in the body weights resulting from a reduction of body fat and/or extracellular body fluid.

Histopathology: No compound related microscopic lesions were observed in any of the tissues from rats that were examined from the 2500 ppm group. Microscopic lesions seen were considered spontaneous and incidental in nature.

Statistical Analysis: All statistical analyses compared the treatment groups with the control group by sex. Body weights (week 13), food consumption (week 13), hematological, biochemical and urinalysis parameters (1, 2 and 3 months) and absolute and relative organ weights (terminal sacrifice) were compared by analysis or variance (one-way classification) Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

Steel, R.G.D. and Torrie, J.H. Principles and Procedures of Statistics. McGraw-Hill. 1960.

Dunnett, C.W. New Tables for Multiple Comparisons with a Control. Biometrics. 1964.

Interpretation/Conclusion: Mid and high dose males and all three groups of treated females had decreased group mean body weights when compared with controls. Mid and high dose males and high dose females had decreased food consumption over the 90-day study when compared with controls. Males and females treated at 2500 ppm had elevated serum alkaline phosphatase values at 1, 2 and 3 months of study. Statistically significant decreases in the mean absolute weights of hearts of male rats ($p<0.05$) and in the mean absolute and relative weights of livers of male and female rats ($p<0.01$) at all dosage levels, appeared to be treatment related. No compound-related gross lesions were seen in any of the rats from the treatment groups. No compound-related microscopic lesions were seen in any of the tissues from rats that were examined from the 2500 ppm group.

Data Quality: 2, Valid with restriction

References: Chlorendic Anhydride, Technical. 90-Day Subacute Toxicity Study in Rats. International Research and Development Corporation. 1980.

DEVELOPMENTAL TOXICITY

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 93.81%

Method: Mating was initiated on February 13, 1978 and the last cesarean section was performed on March 10, 1978. Male rats of the same strain were used for mating. Bred females were consecutively assigned to 4 groups in a block design, one control and 3 treatment groups of 25 rats per group. Prior to compound administration, females were observed daily for mortality and overt signs of toxicity. The females were observed daily for changes in appearance, behavior, mortality and clinical signs of toxicity from gestation days 6 through 20. Individual female body weights were recorded on days 0, 6, 9, 12, 16, and 20 of gestation. On day 20 of gestation, all female rats were sacrificed by an overdose of carbon dioxide, the abdominal and thoracic cavities were examined, and the fetuses delivered by cesarean section. The number and location of viable and nonviable fetuses, early and late resorptions, total implantations and copora lutea were recorded. The sex and body weight were recorded for each fetus. All fetuses were subjected to gross examination to determine sex and any external abnormalities. Approximately one-third of the fetuses were placed in Bouin's fixative and later sectioned by the method described by Wilson to examine viscera. The remaining fetuses were fixed in alcohol, macerated with potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson and examined for skeletal anomalies and variations.

Wilson, J.G. "Embryological Considerations in Teratology." Teratology Principles and Techniques. The University of Chicago Press. 1965.

Dawson, A.B. "Note on the Staining of the Skeleton of Cleared Specimens with Alizarin Red S." Stain Technology. 1926.

GLP: No

Year Performed: 1978

Laboratory: International Research and Development Corporation

Species/Strain: Rat, Charles River CD

Route of Administration: Gastric intubation

Dosages: 0, 25, 100 and 400 mg/kg/day

Number and Sex: 25 pregnant females / group

Exposure period: Days 6 – 19 of gestation

Frequency of Treatment: Daily

Control Group: Corn oil vehicle at a dose of 10 ml/kg/day

Duration of Test: Necropsy on day 20 of gestation

Statistical Evaluation: All statistical analyses compared the treatment groups with the control group, with the level of significance at $p<0.05$. Male to female fetal sex ratio, number of litters with anomalies and number of fetuses with anomalies were compared using the Chi-square test criterion with Yates correction for 2 x 2 contingency tables and/or Fisher's exact probability test as described by Siegel to judge significance of differences. The proportion of early and late resorbed fetuses, nonviable fetuses and postimplantation losses were compared by the Mann Whitney U-test as described by Siegel and Weil to judge significance of differences. Mean number of corpora lutea, total implantations and viable fetuses were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison table to judge significance of differences. Fetal body weights were compared by analysis of variance (hierarchical classification) and t-test as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

Dunnett, C.W. New Tables for Multiple Comparisons with a Control. Biometrics. 1964.

Siegel, S. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill. 1956.

Steel, R.G.D. and Torrie, J.H. Principles and Procedures of Statistics. McGraw-Hill. 1960.

Weil, C.S. Selections of the valid number of sampling units and a consideration of their combination in toxicological studies involving reproduction, teratogenesis or carcinogenesis. Food Cosmetic Toxicology. 1970.

Age at Study Initiation: Approximately 3 months old

Test Substance Preparation: Chlorendic anhydride was suspended, using a tissue homogenizer, in Mazola® corn oil at varying concentrations to permit the administration of 10 ml/kg/day at dosage levels

of 25, 100, and 400 mg/kg/day. Compound administration was by gavage from day 6 through day 15 of gestation. The control females were given the vehicle on a comparable regimen at 10 ml/kg/day. Individual doses were based upon the body weights on gestation days 6, 9 and 12.

Clinical Observation (Maternal): Survival was 100% for all groups. There was a slight loss in mean body weight during the first 3 days of treatment and reduced mean body weight gains throughout the entire treatment period for 400 mg/kg/day dosage group when compared to the control group. Mean body weight gains for the 25 and 100 mg/kg/day dosage groups were comparable to the control. There were no changes in appearance or behavior for the females in the 25 and 100 mg/kg/day dosage group when compared to the rats in the control group. Matted fur, anogenital staining and red nasal discharge were seen for some rats in all groups, however there was a slight increase in the 400 mg/kg/day dosage group when compared to the rats in the control group.

Mating Procedure: Male rats of the same strain were used for mating. One female and one male were placed together for mating. The day of mating was determined by daily inspection for a copulatory plug or vaginal smear for sperm. The day when a plug or sperm was found, was designated day 0 of gestation and the female was returned to an individual cage.

Results

Terminal Observations (Maternal): There were no biologically meaningful or statistically significant differences in the mean number of viable or nonviable fetuses, corpora lutea or in the mean fetal body weight for rats in the 25, 100, or 400 mg/kg/day dosage groups when compared to the control group. The male to female sex ratio in the 25 mg/kg/day dosage group was statistically significantly different from the control group, this difference is attributable to random occurrence and not considered compound related. The increases in the mean number of postimplantation losses in the 100 and 400 mg/kg/day dosage group was statistically significant when compared to the control vehicle. However, this increase is only slightly higher then the mean for the historical control.

Fetal Examination: There were no malformed fetuses in the control group, one malformed fetus in the 25 mg/kg/day group, 2 malformed fetuses in the 100 mg/kg/day and one malformed fetus in the 400 mg/kg/day group. These malformations were not statistically significant and not considered to be treatment related. The variations observed were similar for all groups.

Conclusions:	The number of developmental or genetic variations were comparable for all chlorendic anhydride dosage groups and the control group. The increase in malformations in the chlorendic anhydride groups were not biologically meaningful when compared to the control group. Chlorendic anhydride is not considered teratogenic in rats in dosage levels up to and including 400 mg/kg/day.
Reliability:	2, Valid with restriction
Reference:	Chlorendic Anhydride. Teratology Study in Rats. International Research and Development Corporation. 1978.

REPRODUCTIVE TOXICITY

A reproductive study was not performed for this compound. However, the repeated dose, 90-day study referenced in this robust summary contains adequate data to act as a surrogate for a reproductive study. Specifically, the subacute oral toxicity study included evaluation of the testes as required to meet the OECD/SIDS program requirements.

AMES MUTAGENICITY

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 93.81%

Solvent: Either deionized water or dimethylsulfoxide (DMSO)

Study Type: Bacterial reverse mutation assay

Test Method: Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37°C, and scored for the number of colonies growing on each plate. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

GLP: No

Year Performed: 1977

Laboratory: Litton Bionetics Incorporated

Species/Strain: *Salmonella typhimurium:* TA-1535, TA-1537, TA-1538, TA-98, TA-100

Saccharomyces cerevisiae: D4

Concentrations: 0.1, 1.0, 10, 100, 500 μg / plate

Metabolic Activation: Sprague-Dawley adult male rat liver

Quantity of Activator: 0.5 ml

Induction: Induced by Aroclor 1254

Criteria for Evaluating Results:
Strains TA-1535, TA-1537, and TA-1538: If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

Strains TA-98, TA-100 and D4: If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

Pattern: Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in non-activation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

Reproducibility: If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

Positive Control Chemicals: Nonactivation:
Methylnitrosoguanidine in water or saline at 10 μg/plate
2-Nitrofluorene in DMSO at 100 μg/plate
Quinacrine Mustard in water or saline at 10 μg/plate

Activation:
2-Anthramine in DMSO at 100 μg/plate
2-Acetylaminofluorene in DMSO at 100 μg/plate
8-Aminoquinoline in DMSO at 100 μg/plate

Results: The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats.

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.1 μg to 500 μg per plate.

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

The results of the tests conducted on the compound in the presence of the rat liver assay system were all negative.

Conclusion: Chlorendic anhydride did not demonstrate mutagenic activity in any of the assays conducted in this evaluation.

Data Quality: 2, Valid with restriction

References: Mutagenicity Evaluation of Chlorendic Anhydride. Litton Bionetics Incorporated. 1977.

MAMMALIAN CELL GENE MUTATION

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 93.81%

Study Type: Mouse lymphoma forward mutation assay

Test Method: Non-activation assay: The procedure used is a modification of that reported by Clive and Spector (1975). Prior to each treatment, cells were cleansed of spontaneous TK-/- by growing them in a medium containing thymidine, hypoxanthine, methotrexate, and those cells that produce TK, and can therefore utilize the exogenous thymidine from the medium. The test compound was added to the cleansed cells in growth medium at the predetermined doses for 4 hours. The mutagenized cells were washed, fed and allowed to express in growth medium for 3 days. At the end of this expression period, TK-/- mutants were detected by cloning the cells in the selection medium for 10 days. Surviving cell populations were determined by plating diluted aliquots in nonselective growth medium.

Activation assay: The activation assay differs from the non-activation assay in only the following manner: Two milliliters of the reaction mixture were added to 10 ml growth medium containing the desired number of cleansed cells. After adding the test compound, the flask was incubated on a rotary shaker for 4 hours. The incubation period was terminated by washing the cells twice with growth medium. The washed treated cells were then allowed to express for 3 days and were cloned as indicated for the non-activated cells.

Clive, D. and Spector JFS. Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. *Mutation Res.* 31. 1975.

GLP: No

Year Performed: 1978

Laboratory: Litton Bionetics Incorporated

Species/Strain: Fisher mouse lymphoma L5178Y

Concentrations: Trial One
Nonactivation: 0.06, 0.08, 0.12, 0.16, 0.24 mg/ml
Activation: 0.08, 0.12, 0.16, 0.24, 0.32 mg/ml

Trial Two
Activation: 0.24, 0.32, 0.40, 0.48, 0.56, 0.64 mg/ml

Solvent: 1% DMSO

Metabolic Activation: Mouse liver

Quantity of Activator: 0.5 μg/ml

Induction: Ethylmethanesulfonate (EMS) and Dimethylnitrosamine (DMN)

Criteria for Evaluating Results: A compound is considered mutagenic in this assay if:

- A dose-response relationship is observed over 3 of the 4 dose levels employed
- The minimum increase at the high level of the dose-response curve is at least 2.5 times greater than the solvent and/or negative control values
- The solvent and negative control data are within the normal range of the spontaneous background for the TK locus
- All evaluations of mutagenic activity are based on consideration of the concurrent solvent and negative control values run with the experiment in

question. Positive control values are not used as reference points, abut are included to ensure that the current cell population responds to direct and promutagens under the appropriate treatment conditions.

- Occasionally, a single point within a concentration range will show an increase 2.5 times greater than the spontaneous background. If the increase is at the high dose, is reproducible, and if an additional higher dose level is not feasible because of toxicity, the chemical can be considered mutagenic. If the increase is internal within the dose range and is not reproducible, the increase will normally be considered aberrant. If the internal increase is reproducible, several doses clustered around the positive concentration will be examined to either confirm or reject the reliability of the effect.

Positive/Negative Controls: Negative control: mouse liver tissue
Positive control: Ethylmethanesulfonate (EMS) was dissolved in culture medium for the nonactivation studies at a final concentration of 0.5 μg/ml. Dimethylnitrosamine (DMN) was used as a positive control substance for the activation studies at a final concentration of 0.5 μg/ml.

Results: Chlorendic anhydride was soluble in DMSO at 500 mg/ml at 37°C. For the experiment a stock solution of 32 mg/ml was diluted serially in 2-fold steps with DMSO to provide a series of working solutions. One-tenth milliliter of each working solution was added to 10 ml of growth medium containing 3×10^5 cells/ml to provide the proper final concentration. Some precipitate formed at concentrations of 160 μg/ml and higher in the presence of culture medium but went into solution after mild agitation.

Toxicity was monitored by observations on cell morphology and by changes in cell population growth during the three days of expression. Toxicity was observed at 240 μg/ml with out activation and above 320 μg/ml in the presence of an S9 activation system.

DMSO was used as the solvent control substance. Growth medium without the addition of solvent was employed as a negative control. No genetic effects were attributed to the presence of the solvent. EMS and Dimethylnitrosamine were used as reference mutagens and induced mutation frequencies within the expected range.

No evidence of induced mutagenesis was observed in chlorendic anhydride in either Trial 1 or 2. Trial 2 was initiated in an attempt to observe the response of the cells to a concentration of compound that caused at least a 50% reduction in cell survival. Because of quenching by the S9 liver preparation, it was possible to test concentrations twice those used in the nonactivation series without the induction of mutagenesis.

Interpretation/ Conclusion: Chlorendic anhydride did not induce forward mutation at the TK locus in L5178Y mouse lymphoma cells under the conditions of this assay.

Data Quality: 2, Valid with restriction

References: Mutagenicity Evaluation of Chlorendic Anhydride in the Mouse Lymphoma Forward Assay. Litton Bionetics Incorporated. 1978.

MAMMALIAN CHROMOSOME ABERRATION TEST

Test Substance: Chlorendic anhydride is a white crystalline solid

Purity Profile: 93.81%

Study Type: Mouse Dominant Lethal Assay
Chromosome aberrations including breaks, rearrangements and deletions are believed to produce the dominant lethality. Evidence of dominant lethality emphasized that the compound is able to reach developing germ cells and induce genetic damage. It also suggests, but does not measure directly, that in addition to the detected gross chromosomal lesions, more subtle balanced lesions or specific locus gene mutations may be produced. These latter types have a good chance of being transmitted to the gene pool of future offspring.

Test Method: Ten random bred male mice from a closed colony were assigned to 1 of 5 groups. Three of these groups received different dose levels of the test compound; a fourth group received only the solvent or vehicle; and the fifth group received a known mutagen and served as the positive control group. Triethylenemelamine (TEM) was used as the positive control and was given as a single intraperitoneal injection. Following treatment, each male was rested for 2 days and then caged with 2 unexposed virgin females on the third day. At the end of 5 days, these females were removed. This weekly mating sequence was continued for 7 weeks. Each pair of mated females was transferred to a fresh cage, and approximately 14 days after the midweek of being caged with the male, the females were killed with CO_2. At necropsy, their uteri were examined for dead and living implants and total implantations.

GLP: No

Year Performed: 1978

Laboratory: Litton Bionetics Incorporated

Species/Strain: Adult male and female mice, CD-1 strain

Solvent: DMSO

Doses: 5, 1, 0.5, 0.1, and 0.05 g/kg

Positive Control: Positive: Triethylenemelamine (TEM)

Results:

	Date	Dosage	Deaths / 6 Animals Day													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	9/30/77	0.3 ml 5 g/kg	1	0	5	0	0	0	0	0	0	0	0	0	0	0
2	9/30/77	0.3 ml 1 g/kg	1	0	0	1	0	0	1	0	0	0	0	0	0	0
3	9/30/77	0.03 ml 0.5 g/kg	0	0	0	0	1	0	0	0	0	0	1	0	0	0
4	9/30/77	0.03 ml 0.1 g/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	9/30/77	0.03 ml 0.05 g/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	9/30/77															

Calculated Values:

LD_{50}: 0.835	LD_1: 0.129	LD_5: 0.223	
Upper 2.610	Upper 0.322	Upper 0.455	Slope 2.8705
Lower 0.361	Lower 0.000	Lower 0.001	Intercept 5.2241

Chlorendic anhydride was examined for its ability to induce dominant lethality in male mice. The test material was evaluated at 0.223 g/kg, 0.074 g/kg and 0.022 g/kg. The vehicle for this test was DMSO and the route of administration was oral. Following dosing, the males were mated sequentially for 7 weeks to virgin female mice. Pregnant females were scored for dominant lethal indexes at mid-pregnancy.

The data did not indicate any evidence of compound-induced dominant lethality. Some reduction in fertility was observed at week 5 but none of the other parameters produced significant trends during the same week.

The positive control was active over the first three mating weeks, indicating induction of alterations in sperm and spermatids.

Conclusion: Chlorendic anhydride was considered to be inactive in this test and did not induce dominant lethality in mice under the test conditions employed in this evaluation.

Data Quality: 2, Valid with restriction

References: Mutagenicity Evaluation of Chlorendic Anhydride in the Mouse Dominant Lethal Assay. Litton Bionetics Incorporated. 1978.